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(54) Title: USE OF COLOSTRININ, CONSTITUENT PEPTIDES THEREOF, AND ANALOGS THEREOF FOR INDUCING CYTOKINES

(57) Abstract: The present invention discloses a use of colostrinin, a constituent peptide thereof, and/or an analog thereof as an immunological regulator adn as a blood cell regulator in animals including humans.

USE OF COLOSTRININ, CONSTITUENT PEPTIDES THEREOF, AND ANALOGS THEREOF FOR INDUCING CYTOKINES

Background of the Invention

Colostrum is a component of the milk of mammals during the first few days after birth. Colostrum is a thick yellowish fluid and is the first lacteal secretion post parturition and contains a high concentration of immunogloblins (IgG, IgM, and IgA) and a variety of non-specific proteins. Colostrum also contains various cells such as granular and stromal cells, neutrophils, monocyte/macrophages, and lymphocytes. Colostrum also includes growth factors, hormones, and cytokines. Unlike mature breast milk, colostrum contains low sugar, low iron, but is rich is lipids, proteins, mineral salts, vitamins, and immunoglobins.

Colostrum also includes or contains a proline-rich polypeptide aggregate, which is referred to as colostrinin. One peptide fragment of colostrinin is Val-Glu-Ser-Tyr-Val-Pro-Leu-Phe-Pro (SEQ ID NO:31), which is disclosed in International Publication No. WO-A-98/14473. Colostrinin and this fragment have been identified as useful in the treatment of disorders of the central nervous system, neurological disorders, mental disorders, dementia, neurodegenerative diseases, Alzheimer's disease, motor neurone disease, psychosis, neurosis, chronic disorders of the immune system, diseases with a bacterial and viral aetiology, and acquired immunological deficiencies as set forth in International Publication No. WO-A-98/14473.

Although certain uses for colostrinin have been identified, it would represent an advancement in the art to discover and disclose other uses for colostrinin, or a component thereof, that are not readily ascertainable from the information currently known about colostrinin or its constituents.

SUMMARY OF THE INVENTION

The present invention relates to the use of colostrinin, at least one constituent (i.e., component) peptide thereof, at least one active analog thereof (e.g., peptide having an N-terminal sequence equivalent to an N-terminal sequence of at least one of the colostrinin constituent peptides), and combinations thereof, as a cytokine-inducing agent. These agents can be used as immunological regulators to modulate (e.g., enhance, inhibit, modify, augment, or otherwise alter, and preferably promote) specific or nonspecific immune responses in patients, particularly animals including mammals such as humans. They can also be used as blood cell regulators to modulate (e.g., enhance, inhibit, modify, augment, or otherwise alter, preferably, and promote) cellular proliferation or differentiation (preferably, promoting proliferation and differentiation) of blood cells, such as leukocytes.

In one embodiment, the present invention provides a method of inducing a cytokine in a cell. The method includes contacting the cell with an immunological regulator under conditions effective to induce (i.e., induce the synthesis or production of) at least one cytokine (either directly or indirectly), wherein the immunological regulator is selected from the group of MQPPPLP (SEQ ID NO:1); LQTPQPLLQVMMEPQGD (SEQ ID NO:2); DQPPDVEKPDLQPFQVQS (SEQ ID NO:3); LFFFLPVVNVLP (SEQ ID NO:4); DLEMPVLPVEPFPFV (SEQ ID NO:5); MPQNFYKLPQM (SEQ ID NO:6); VLEMKFPPPPQETVT (SEQ ID NO:7); LKPFPKLKVEVFPFP (SEQ ID NO:8); VVMEV (SEQ ID NO:9); SEQP (SEQ ID NO:10); DKE (SEQ ID NO:11); FPPPK (SEQ ID NO:12); DSQPPV (SEQ ID NO:13); DPPPPQS (SEQ ID NO:14); SEEMP (SEQ ID NO:15); KYKLQPE (SEQ ID NO:16); VLPPNVG (SEQ ID NO:17); VYPFTGPIPN (SEQ ID NO:18); SLPQNILPL (SEQ ID NO:19); TQTPVVVPPF (SEQ ID NO:20); LQPEIMGVPKVKETMVPK (SEQ ID NO:21); HKEMPFPKYPVEPFTESQ (SEQ ID NO:22); SLTLTDVEKLHLPLPLVQ (SEQ ID NO:23); SWMHQPP (SEQ ID NO:24); QPLPPTVMFP (SEQ ID NO:25); PQSVLS (SEQ ID NO:26); LSQPKVLPVPQKAVPQRDMPIQ (SEQ ID NO:27); AFLLYQE (SEQ ID NO:28); RGPFPILV (SEQ ID NO:29); ATFNRYQDDHGEEILKSL (SEQ ID

NO:30); FLLYQEPVLGPVR (SEQ ID NO:32); LNF (SEQ ID NO:33); and MHQPPQPLPPTVMFP (SEQ ID NO:34); an active analog thereof; and combinations thereof; with the proviso that the immunological regulator is not VESYVPLFP (SEQ ID NO:31). The cell can be in a cell culture, a tissue, an organ, or an organism. Hence, this method can be carried out *in vivo* or *in vitro*.

In another embodiment, there is provided a method for modulating an immune response in a cell. The method includes contacting the cell with an immunological regulator under conditions effective to induce at least one cytokine, wherein the immunological regulator is listed above. The cell can be in a cell culture, a tissue, an organ, or an organism. Hence, this method can be carried out *in vivo* or *in vitro*.

In yet another embodiment, there is provided a method for modulating an immune response in a patient. The method includes administering to the patient an immunological regulator under conditions effective to induce at least one cytokine, wherein the immunological regulator is listed above.

The immune response can be specific or nonspecific. Typically, one or more cytokines are directly induced using the polypeptides described herein, which then results in an upregulation or a downregulation of one or more other cytokines. Thus, using various combinations of polypeptides described herein, various cytokine profiles and immune responses can be produced, which may be specific or nonspecific. Examples of such immune responses include the interferon response and antibody production. As long as at least one cytokine level increases, whether it be increased as a result of direct inducement by one of the peptides described herein, or as a result of indirect inducement (e.g., through the interaction with another cytokine), a peptide is "active" as used herein.

In another embodiment, there is provided a method for modulating blood cell proliferation. The method includes contacting blood cells with a blood cell regulator selected from the group of colostrinin, a constituent peptide thereof, an analog thereof, and combinations thereof, under conditions effective to change the number of blood cells. The blood cells can be in a cell culture or an organism. Hence, this method can be carried out *in vivo* or *in vitro*.

In still another embodiment, there is provided a method for modulating

blood cell proliferation in a patient (preferably, a human patient). The method includes administering to the patient a blood cell regulator selected from the group of colostrinin, a constituent peptide thereof, an analog thereof, and combinations thereof, under conditions effective to change the number of blood cells.

The blood cells can be mammalian blood cells, such as human blood cells. Preferably, the blood cells are increased in number, although a decrease in number can also be desirable in certain situations such as leukemia, myelopathy, etc. More preferably, the blood cells are increased in number and differentiated. The blood cell regulator is preferably a constituent peptide of colostrinin.

In other embodiments, the invention provides the use of an immunological regulator or blood cell regulator in the manufacture of a medicament for use in the methods described herein.

The present invention also provides an immune-inducing composition that includes a pharmaceutical carrier and an active agent selected from the MQPPPLP (SEQ ID NO:1); LQTPQPLLQVMMEPQGD (SEQ ID NO:2); DQPPDVEKPDLQPFQVQS (SEQ ID NO:3); LFFFLPVVNVLP (SEQ ID NO:4); DLEMPVLPVEPFPFV (SEQ ID NO:5); MPQNFYKLPQM (SEQ ID NO:6); VLEMKFPPPPQETVT (SEQ ID NO:7); LKPFPKLKVEVFPFP (SEQ ID NO:8); VVMEV (SEQ ID NO:9); SEQP (SEQ ID NO:10); DKE (SEQ ID NO:11); FPPPK (SEQ ID NO:12); DSQPPV (SEQ ID NO:13); DPPPPQS (SEQ ID NO:14); SEEMP (SEQ ID NO:15); KYKLQPE (SEQ ID NO:16); VLPPNVG (SEQ ID NO:17); VYPFTGPIPN (SEQ ID NO:18); SLPQNILPL (SEQ ID NO:19); TQTPVVVPPF (SEQ ID NO:20); LQPEIMGVPKVKETMVPK (SEQ ID NO:21); HKEMPFPKYPVEPFTESQ (SEQ ID NO:22); SLTLTDVEKLHLPLPLVQ (SEQ ID NO:23); SWMHQPP (SEQ ID NO:24); QPLPPTVMFP (SEQ ID NO:25); PQSVLS (SEQ ID NO:26); LSQPKVLPVPQKAVPQRDMPIQ (SEQ ID NO:27); AFLLYQE (SEQ ID NO:28); RGPFPILV (SEQ ID NO:29); ATFNRYQDDHGEEILKSL (SEQ ID NO:30); FLLYQEPVLGPVR (SEQ ID NO:32); LNF (SEQ ID NO:33); and MHQPPQPLPPTVMFP (SEQ ID NO:34); an active analog thereof; and combinations thereof; with the proviso that the immunological regulator is not

VESYVPLFP (SEQ ID NO:31).

As used herein, "a" or "an" means one or more (or at least one), such that combinations of active agents (i.e., active immunological regulators or blood cell differentiation promoters), for example, can be used in the compositions and methods of the invention. Thus, a composition that includes "a" polypeptide refers to a composition that includes one or more polypeptides.

"Amino acid" is used herein to refer to a chemical compound with the general formula: NH_2---CRH---COOH, where R, the side chain, is H or an organic group. Where R is organic, R can vary and is either polar or nonpolar (i.e., hydrophobic). The amino acids of this invention can be naturally occurring or synthetic (often referred to as nonproteinogenic). As used herein, an organic group is a hydrocarbon group that is classified as an aliphatic group, a cyclic group or combination of aliphatic and cyclic groups. The term "aliphatic group" means a saturated or unsaturated linear or branched hydrocarbon group. This term is used to encompass alkyl, alkenyl, and alkynyl groups, for example. The term "cyclic group" means a closed ring hydrocarbon group that is classified as an alicyclic group, aromatic group, or heterocyclic group. The term "alicyclic group" means a cyclic hydrocarbon group having properties resembling those of aliphatic groups. The term "aromatic group" refers to mono- or polycyclic aromatic hydrocarbon groups. As used herein, an organic group can be substituted or unsubstituted.

The terms "polypeptide" and "peptide" are used interchangeably herein to refer to a polymer of amino acids. These terms do not connote a specific length of a polymer of amino acids. Thus, for example, the terms oligopeptide, protein, and enzyme are included within the definition of polypeptide or peptide, whether produced using recombinant techniques, chemical or enzymatic synthesis, or naturally occurring. This term also includes polypeptides that have been modified or derivatized, such as by glycosylation, acetylation, phosphorylation, and the like.

The following abbreviations are used throughout the application:

A = Ala = Alanine	T = Thr = Threonine
V = Val = Valine	C = Cys = Cysteine
L = Leu = Leucine	Y = Tyr = Tyrosine
I = Ile = Isoleucine	N = Asn = Asparagine
P = Pro = Proline	Q = Gln = Glutamine
F = Phe = Phenylalanine	D = Asp = Aspartic Acid
W = Trp = Tryptophan	E = Glu = Glutamic Acid
M = Met = Methionine	K = Lys = Lysine
G = Gly = Glycine	R = Arg = Arginine
S = Ser = Serine	H = His = Histidine

Detailed Description of Preferred Embodiments of the Invention

The inventors have found that colostrinin, at least one constituent (i.e., component) peptide thereof, and/or at least one active analog thereof (e.g., a peptide having an N-terminal sequence equivalent to an N-terminal sequence of at least one of the colostrinin constituent peptides) can be used to induce at least one cytokine (e.g., TNF-α, IFN-γ, IL-1, IL-2, IL-4, IL-6, I-10, IL-12). The cytokine can be either directly or indirectly induced. This can result in the modulation of an immune response or blood cell proliferation or differentiation (preferably, the promotion of blood cell proliferation, and more preferably, the promotion of blood cell proliferation and differentiation) *in vitro* and *in vivo*, in animals (including mammals such as humans).

Such immunological regulators and blood cell regulators are referred to herein as "active agents." Significantly, such agents can be administered alone or in various combinations to a patient (e.g., animals including humans) as a medication or dietary (e.g., nutrient) supplement in a dose sufficient to modulate one or more immune responses throughout the patient's body, in a specific tissue site, or in a collection of tissue sites.

Many nonspecific and specific immune responses are associated with leukocyte proliferation and differentiation. The overall immunological

significance of the present invention can be, but is not limited to, the following: IFN-γ is a potent immunomodulater that is important for the development of the cytotoxic lymphocyte response (CTL). This immune response is considered to be very important in protecting humans and animals from a variety of bacterial, viral, parasitic, and fungal diseases. The fact that TNF-α is also induced is important because TNF-α is a major activator of macrophages, among other immune cells, which are important in host defense against infections. In addition, TNF-α has been shown to have activity against cancer, directly through its lytic activity and indirectly through macrophages. IL-10 is another important immune mediator that controls both IFN-γ and TNF-α production and action. Its production represent a negative feedback control for IFN-γ and TNF-α production. Another one of its hallmark activities is the control of antibody production during the humoral immune responses, which is certainly important in many types of infections. In addition to IL-10's immune activities, it also has been shown to play a role in the neuroendocrine system by modulating certain stress responses and immune responses. IL-10 has been shown to induce the production of corticotropin from pitutitary cells. Corticotropin works downstreanm in the hypothalmic adrenal axis to induce glucocortico steroids that are inherently immunomodulatory. Like IL-10, the IL-4 is important in the development of B cell responses, which are the mediators of the humoral immune response. Finally, the IL-12 is an important IFN-γ inducer. Taken together these findings suggest that colostrinin and its component peptides have the ability to modulate via cytokine induction a variety of host-defense mechanisms mediated by macrophages and lymphocytes at the cellular and humoral immune level as well as the neuroendocrine system.

Thus, the methods and compositions of the present invention can be utilized to control immunological and blood cell differentiating activity. The active agents described herein can be used individually, in various combinations, or combined with other previously known or newly invented pharmacological agents, such as antioxidants. They can be used as adjuvants for existing vaccinations as well.

In a preferred embodiment, the present invention provides a method for

modulating an immune response. Whether it be *in vivo* or *in vitro*, this method involves monitoring the level of at least one cytokine, which can be done by known methods, such as disclosed by Inglot et al., Arch. Immunol. Ther. Exp., 44, 215-224 (1996); Blach-Olszewska et al., Arch. Immunol. Ther. Exp., 45, 43-47 (1997); Piasecki et al., Arch. Immunol. Ther. Exp., 45, 109-117 (1997); Hughes et al., Int. J. Immunopharmacol., 17, 857-863 (1995); and Mishell et al., Selected Methods in Cellular Immunology, W.H. Freeman, 1980. Specific *in vitro* methods are described in the Examples Section.

In another preferred embodiment, the present invention provides a method for modulating blood cell proliferation (preferably, proliferation and differentiation). Whether it be *in vivo* or *in vitro*, this method involves monitoring the level of increase or decrease in the number of blood cells bearing a specific phenotypic marker (for differentiation, the types of cells formed are evaluated), as disclosed by Kim et al., Clin. Lab. Haematol., 20, 21-29 (1998); Grunwald et al., Methods Mol. Biol., 119, 443-454 (1999); Villas et al., Cell. Vis., 5, 56-61 (1998); and Gratama et al., Cytometry, 33, 166-178 (1998). Specific *in vitro* methods are described in the Examples Section.

The peptides described herein may be used for the proliferation and/or differentiation of other types of cells as well.

Colostrinin is composed of peptides, the aggregate of which has a molecular weight range between about 5.8 to about 26 kiloDaltons (kDa) determined by polyacrylamide gel electrophoresis. It has a greater concentration of proline than any other amino acid. Ovine colostrinin has been found to have a molecular weight of about 18 kDa and includes three non-covalently linked subunits having a molecular weight of about 6 kDa and has about 22 wt-% proline. Ovine colostrinin has also been shown to contain the following number of residues per subunit: lysine - 2; histidine - 1; arginine - 0; aspartic acid - 2; threonine - 4; serine - 3; glutamic acid - 6; proline - 11; glycine - 2; alanine - 0; valine - 5; methionine - 2; isoleucine - 2; leucine - 6; tyrosine - 1; phenylalanine - 3; and cysteine - 0.

Colostrinin has been found to include a number of peptides ranging from 3 amino acids to 22 amino acids or more. These can be obtained by various

known techniques, including isolation and purification involving eletrophoresis and synthetic techniques. The specific method of obtaining colostrinin and SEQ ID NO:31 is described in International Publication No. WO-A-98/14473. Using HPLC and Edelman Degradation, over 30 constituent peptides of colostrinin have been identified, which can be classified into several groups: (A) those of unknown precursor; (B) those having a β-casein homologue precursor; (C) those having a β-casein precursor; and (D) those having an annexin precursor. These peptides are described in International Patent Application PCT/GB00/02128, filed June 2, 2000, claiming priority to June 2, 1999, and can be synthesized according to the general method described in the Examples Section. These peptides (i.e., constituent peptides of colostrinin), which can be derived from colostrinin or chemically synthesized, include: MQPPPLP (SEQ ID NO:1); LQTPQPLLQVMMEPQGD (SEQ ID NO:2); DQPPDVEKPDLQPFQVQS (SEQ ID NO:3); LFFFLPVVNVLP (SEQ ID NO:4); DLEMPVLPVEPFPFV (SEQ ID NO:5); MPQNFYKLPQM (SEQ ID NO:6); VLEMKFPPPPQETVT (SEQ ID NO:7); LKPFPKLKVEVFPFP (SEQ ID NO:8); VVMEV (SEQ ID NO:9); SEQP (SEQ ID NO:10); DKE (SEQ ID NO:11); FPPPK (SEQ ID NO:12); DSQPPV (SEQ ID NO:13); DPPPPQS (SEQ ID NO:14); SEEMP (SEQ ID NO:15); KYKLQPE (SEQ ID NO:16); VLPPNVG (SEQ ID NO:17); VYPFTGPIPN (SEQ ID NO:18); SLPQNILPL (SEQ ID NO:19); TQTPVVVPPF (SEQ ID NO:20); LQPEIMGVPKVKETMVPK (SEQ ID NO:21); HKEMPFPKYPVEPFTESQ (SEQ ID NO:22); SLTLTDVEKLHLPLPLVQ (SEQ ID NO:23); SWMHQPP (SEQ ID NO:24); QPLPPTVMFP (SEQ ID NO:25); PQSVLS (SEQ ID NO:26); LSQPKVLPVPQKAVPQRDMPIQ (SEQ ID NO:27); AFLLYQE (SEQ ID NO:28); RGPFPILV (SEQ ID NO:29); ATFNRYQDDHGEEILKSL (SEQ ID NO:30); VESYVPLFP (SEQ ID NO:31); FLLYQEPVLGPVR (SEQ ID NO:32); LNF (SEQ ID NO:33); and MHQPPQPLPPTVMFP (SEQ ID NO:34). These can be classified as follows: (A) those of unknown precursor include SEQ ID NOs:2, 6, 7, 8, 10, 11, 14, and 33; (B) those having a β-casein homologue precursor include SEQ ID NOs:1, 3, 4, 5, 9, 12, 13, 15, 16, 17, and 31; (C) those having a β-casein precursor include SEQ ID NOs:18 (casein amino

acids 74-83), 19 (casein amino acids 84-92), 20 (casein amino acids 93-102), 21 (casein amino acids 103-120), 22 (casein amino acids 121-138), 23 (casein amino acids 139-156), 24 (casein amino acids 157-163), 25 (casein amino acids 164-173), 26 (casein amino acids 174-179), 27 (casein amino acids 180-201), 28 (casein amino acids 202-208), 29 (casein amino acids 214-222), 32 (casein amino acids 203-214), and 34 (casein amino acids 159-173); and (D) those having an annexin precursor include SEQ ID NO:30 (annexin amino acids 203-220).

For certain embodiments, a preferred group of such peptides does not include SEQ ID NO:31. A more preferred group of such peptides includes: MQPPPLP (SEQ ID NO:1); LQTPQPLLQVMMEPQGD (SEQ ID NO:2); DQPPDVEKPDLQPFQVQS (SEQ ID NO:3); LFFFLPVVNVLP (SEQ ID NO:4); DLEMPVLPVEPFPFV (SEQ ID NO:5); MPQNFYKLPQM (SEQ ID NO:6); VLEMKFPPPPQETVT (SEQ ID NO:7); LKPFPKLKVEVFPFP (SEQ ID NO:8); VYPFTGPIPN (SEQ ID NO:18), SLPQNILPL (SEQ ID NO:19), TQTPVVVPPF (SEQ ID NO:20), HKEMPFPKYPVEPFTESQ (SEQ ID NO:22), and combinations thereof.

The polypeptides of SEQ ID NOs:1-34 can be in their free acid form or they can be amidated at the C-terminal carboxylate group. The present invention also includes analogs of the polypeptides of SEQ ID NOs:1-34, which includes polypeptides having structural similarity with SEQ ID NOs:1-34. These peptides can also form a part of a larger peptide. An "analog" of a polypeptide includes at least a portion of the polypeptide, wherein the portion contains deletions or additions of one or more contiguous or noncontiguous amino acids, or containing one or more amino acid substitutions. An "analog" can thus include additional amino acids at one or both of the terminii of the polypeptides listed above. Substitutes for an amino acid in the polypeptides of the invention are preferably conservative substitutions, which are selected from other members of the class to which the amino acid belongs. For example, it is well-known in the art of protein biochemistry that an amino acid belonging to a grouping of amino acids having a particular size or characteristic (such as charge, hydrophobicity and hydrophilicity) can generally be substituted for

another amino acid without substantially altering the structure of a polypeptide.

For the purposes of this invention, conservative amino acid substitutions are defined to result from exchange of amino acids residues from within one of the following classes of residues: Class I: Ala, Gly, Ser, Thr, and Pro (representing small aliphatic side chains and hydroxyl group side chains); Class II: Cys, Ser, Thr and Tyr (representing side chains including an -OH or -SH group); Class III: Glu, Asp, Asn and Gln (carboxyl group containing side chains): Class IV: His, Arg and Lys (representing basic side chains); Class V: Ile, Val, Leu, Phe and Met (representing hydrophobic side chains); and Class VI: Phe, Trp, Tyr and His (representing aromatic side chains). The classes also include related amino acids such as 3Hyp and 4Hyp in Class I; homocysteine in Class II; 2-aminoadipic acid, 2-aminopimelic acid, γ-carboxyglutamic acid, β-carboxyaspartic acid, and the corresponding amino acid amides in Class III; ornithine, homoarginine, N-methyl lysine, dimethyl lysine, trimethyl lysine, 2,3-diaminopropionic acid, 2,4-diaminobutyric acid, homoarginine, sarcosine and hydroxylysine in Class IV; substituted phenylalanines, norleucine, norvaline, 2-aminooctanoic acid, 2-aminoheptanoic acid, statine and β-valine in Class V; and naphthylalanines, substituted phenylalanines, tetrahydroisoquinoline-3-carboxylic acid, and halogenated tyrosines in Class VI.

Preferably, active analogs of colostrinin and its constituent peptides include polypeptides having a relatively large number of proline residues. Because proline is not a common amino acid, a "large number" preferably means that a polypeptide includes at least about 15% proline (by number), and more preferably at least about 20% proline (by number). Most preferably, active analogs include more proline residues than any other amino acid. For certain embodiments, preferred group of such active analogs does not include SEQ ID NO:31.

As stated above, active analogs of colostrinin and its constituent peptides include polypeptides having structural similarity. Structural similarity is generally determined by aligning the residues of the two amino acid sequences to optimize the number of identical amino acids along the lengths of their sequences; gaps in either or both sequences are permitted in making the

alignment in order to optimize the number of identical amino acids, although the amino acids in each sequence must nonetheless remain in their proper order. Preferably, two amino acid sequences are compared using the Blastp program, version 2.0.9, of the BLAST 2 search algorithm, available at http://www.ncbi.nlm.nih.gov/gorf/bl2.html. Preferably, the default values for all BLAST 2 search parameters are used, including matrix = BLOSUM62; open gap penalty = 11, extension gap penalty = 1, gap x_dropoff = 50, expect = 10, wordsize = 3, and filter on. In the comparison of two amino acid sequences using the BLAST search algorithm, structural similarity is referred to as "identity." Preferably, an active analog of colostrinin or its constituent peptides has a structural similarity to colostrinin or one or more of its constituent peptides (preferably, one of SEQ ID NOs:1-30) of at least about 70% identity, more preferably, at least about 80% identity, and most preferably, at least about 90% identity.

Colostrinin or any combination of its peptide components or active analogs thereof can be derived (preferably, isolated and purified) naturally such as by extraction from colostrum or can be synthetically constructed using known peptide polymerization techniques. For example, the peptides of the invention may be synthesized by the solid phase method using standard methods based on either t-butyloxycarbonyl (BOC) or 9-fluorenylmethoxy-carbonyl (FMOC) protecting groups. This methodology is described by G.B. Fields et al. in Synthetic Peptides: A User's Guide, W.M. Freeman & Company, New York, NY, pp. 77-183 (1992). Moreover, gene sequence encoding the colostrinin peptides or analogs thereof can be constructed by known techniques such as expression vectors or plasmids and transfected into suitable microorganisms that will express the DNA sequences thus preparing the peptide for later extraction from the medium in which the microorganism are grown. For example, U.S. Patent No. 5,595,887 describes methods of forming a variety of relatively small peptides through expression of a recombinant gene construct coding for a fusion protein which includes a binding protein and one or more copies of the desired target peptide. After expression, the fusion protein is isolated and cleaved using chemical and/or enzymatic methods to produce the desired target peptide.

The peptides used in the methods of the present invention may be employed in a monovalent state (i.e., free peptide or a single peptide fragment coupled to a carrier molecule). The peptides may also be employed as conjugates having more than one (same or different) peptide fragment bound to a single carrier molecule. The carrier may be a biological carrier molecule (e.g., a glycosaminoglycan, a proteoglycan, albumin or the like) or a synthetic polymer (e.g., a polyalkyleneglycol or a synthetic chromatography support). Typically, ovalbumin, human serum albumin, other proteins, polyethylene glycol, or the like are employed as the carrier. Such modifications may increase the apparent affinity and/or change the stability of a peptide. The number of peptide fragments associated with or bound to each carrier can vary, but from about 4 to 8 peptides per carrier molecule are typically obtained under standard coupling conditions.

For instance, peptide/carrier molecule conjugates may be prepared by treating a mixture of peptides and carrier molecules with a coupling agent, such as a carbodiimide. The coupling agent may activate a carboxyl group on either the peptide or the carrier molecule so that the carboxyl group can react with a nucleophile (e.g., an amino or hydroxyl group) on the other member of the peptide/carrier molecule, resulting in the covalent linkage of the peptide and the carrier molecule. For example, conjugates of a peptide coupled to ovalbumin may be prepared by dissolving equal amounts of lyophilized peptide and ovalbumin in a small volume of water. In a second tube, 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide hydrochloride (EDC; ten times the amount of peptide) is dissolved in a small amount of water. The EDC solution was added to the peptide/ovalbumin mixture and allowed to react for a number of hours. The mixture may then dialyzed (e.g., into phosphate buffered saline) to obtain a purified solution of peptide/ovalbumin conjugate. Peptide/carrier molecule conjugates prepared by this method typically contain about 4 to 5 peptides per ovalbumin molecule.

The present invention also provides a composition that includes one or more active agents (i.e., colostrinin, at least one constituent peptide thereof, or active analog thereof) of the invention and one or more carriers, preferably a

pharmaceutically acceptable carrier. The methods of the invention include administering to, or applying to the skin of, a patient, preferably a mammal, and more preferably a human, a composition of the invention in an amount effective to produce the desired effect. The active agents of the present invention are formulated for enteral administration (oral, rectal, etc.) or parenteral administration (injection, internal pump, etc.). The administration can be via direct injection into tissue, interarterial injection, intervenous injection, or other internal administration procedures, such as through the use of an implanted pump, or via contacting the composition with a mucus membrane in a carrier designed to facilitate transmission of the composition across the mucus membrane such as a suppository, eye drops, inhaler, or other similar administration method or via oral administration in the form of a syrup, a liquid, a pill, capsule, gel coated tablet, or other similar oral administration method. The active agents can be incorporated into an adhesive plaster, a patch, a gum, and the like, or it can be encapsulated or incorporated into a bio-erodible matrix for controlled release.

The carriers for internal administration can be any carriers commonly used to facilitate the internal administration of compositions such as plasma, sterile saline solution, IV solutions or the like. Carriers for administration through mucus membranes can be any well-known in the art. Carriers for administration oral can be any carrier well-known in the art.

The formulations may be conveniently presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active agent into association with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active agent into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into the desired formulations.

Formulations suitable for parenteral administration conveniently include a sterile aqueous preparation of the active agent, or dispersions of sterile powders of the active agent, which are preferably isotonic with the blood of the recipient. Isotonic agents that can be included in the liquid preparation include

sugars, buffers, and sodium chloride. Solutions of the active agent can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions of the active agent can be prepared in water, ethanol, a polyol (such as glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, glycerol esters, and mixtures thereof. The ultimate dosage form is sterile, fluid, and stable under the conditions of manufacture and storage. The necessary fluidity can be achieved, for example, by using liposomes, by employing the appropriate particle size in the case of dispersions, or by using surfactants. Sterilization of a liquid preparation can be achieved by any convenient method that preserves the bioactivity of the active agent, preferably by filter sterilization. Preferred methods for preparing powders include vacuum drying and freeze drying of the sterile injectible solutions. Subsequent microbial contamination can be prevented using various antimicrobial agents, for example, antibacterial, antiviral and antifungal agents including parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. Absorption of the active agents over a prolonged period can be achieved by including agents for delaying, for example, aluminum monostearate and gelatin.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as tablets, troches, capsules, lozenges, wafers, or cachets, each containing a predetermined amount of the active agent as a powder or granules, as liposomes containing the active agent, or as a solution or suspension in an aqueous liquor or non-aqueous liquid such as a syrup, an elixir, an emulsion, or a draught. The amount of active agent is such that the dosage level will be effective to produce the desired result in the subject.

Nasal spray formulations include purified aqueous solutions of the active agent with preservative agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal mucous membranes. Formulations for rectal or vaginal administration may be presented as a suppository with a suitable carrier such as cocoa butter, or hydrogenated fats or hydrogenated fatty carboxylic acids. Ophthalmic formulations are prepared by a similar method to the nasal spray, except that the pH and isotonic factors are preferably adjusted to match that of the eye. Topical formulations include the

active agent dissolved or suspended in one or more media such as mineral oil, DMSO, polyhydroxy alcohols, or other bases used for topical pharmaceutical formulations.

Useful dosages of the active agents can be determined by comparing their *in vitro* activity and the *in vivo* activity in animal models. Methods for extrapolation of effective dosages in mice, and other animals, to humans are known in the art; for example, see U.S. Patent No. 4,938,949.

The tablets, troches, pills, capsules, and the like may also contain one or more of the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; an excipient such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; a sweetening agent such as sucrose, fructose, lactose or aspartame; and a natural or artificial flavoring agent. When the unit dosage form is a capsule, it may further contain a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac, or sugar and the like. A syrup or elixir may contain one or more of a sweetening agent, a preservative such as methyl- or propylparaben, an agent to retard crystallization of the sugar, an agent to increase the solubility of any other ingredient, such as a polyhydric alcohol, for example glycerol or sorbitol, a dye, and flavoring agent. The material used in preparing any unit dosage form is substantially nontoxic in the amounts employed. The active agent may be incorporated into sustained-release preparations and devices.

Examples

The invention will be further described by reference to the following detailed examples. The examples are meant to provide illustration and should not be construed as limiting the scope of the present invention. All peptides were dissolved in a balanced salt solution and/or DMSO.

Preparation of Peptides:

1. Wash pre-loaded resin with DMF (dimethylformamide), then drain completely.

2. Add 10 ml of 20% piperidine/DMF to resin. Shake for 5 minutes, then drain.

3. Add another 10 ml of 20% piperidine/DMF. Shake for 30 minutes.

4. Drain reaction vessel and wash resin with DMF four times. Then wash once with DCM (dichloromethanol). Check beads using the ninhydrin test - the beads should be blue.

5. The coupling step was carried out as follows:

   a. Prepare the following solution: 1 mmole Fmoc (i.e. fluorenylmethyloxycarbonyl) amino acid 2.1 ml of 0.45 M HBTU/HOBT (1 mmol) (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate/N-hydroxybenzotriazole-H_2O) 348 µl of DIEA (2 mmol) (diisopropylethylamine); and

   b. Add the solution to the resin and shake for a minimum of 30 minutes.

6. Drain reaction vessel and wash the resin again with DMF four times and with DCM once.

7. Perform the ninhydrin test: If positive (no colour) - proceed to step 2 and continue synthesis; If negative (blue colour) - return to step 5 and recouple the same Fmoc amino acid.

8. After the synthesis was complete, the peptide was cleaved from the resin with 5% H_2O, 5% phenol, 3% Thionisole, 3% EDT (ethanedithiol), 3% triisopropylsilane and 81% TFA for 2 hours.

9. After 2 hours, filter into cold MTBE (methyl t-butyl ether). The precipitated peptide was then washed twice with cold MTBE and dried under nitrogen gas.

10. The molecular weight of the synthesised peptides was checked by Matrix-Assisted Laser Desorption Time-of-Flight Mass Spectroscopy (LDMS), and the purity was checked by HPLC using a C-18, 300 Angstrom, 5 µm column.

Induction of Bood Cell Proliferation: The quantity of peripheral blood leukocyte (PBL) stimulation was determined by measuring the amount of ^{3}H-thymidine (1.0 to 2.0 μC thymidine/culture) incorporated into triplicate cultures (4 x 10^5 PBLs/culture) stimulated with colostrinin and its constituent peptides (CCP) for 72 hours. ^{3}H-thymidine was then added and allowed to incorporate for 24 hours. Staphylococcal enterotoxin A (SEA, also referred to as "super antigen"), a specific T cell mitogen, was used as a positive control and for comparative purposes. Colostrum and low and high iron containing baby formulas diluted 1:5 and 1:10 were also used in some experiments to determine the relative stimulatory activity of these products. Radioactivity was measured in a Matrix 9600 Direct Beta Counter. Six replicas of medium treated cultures were used to determine the mean background incorporated counts. The data is expressed as the mean ^{3}H-thymidine counts per minute (CPM) above background. Results of one out of a total of six experiments are shown below in Table 1.

It can be seen that colostrinin and its constituent peptides are excellent inducers of PBL proliferation. Active concentrations ranged from 100 μg/ml to 0.1 μg/ml. Nine peptides and colostrinin and colostrum were tested. Certain peptides appeared to have greater activity than others with the maximum increase in proliferative activity being roughly 10 times above background. It appears that with many of the peptides, the active range of proliferation induction was present since concentrations as low as 0.1 μg/ml still had potent activity. Some of the peptides had more activity than colostrinin alone. Another interesting finding is that colostrum appears to have roughly an equivalent amount of activity as colostrinin. SEA has the greatest activity and this is also not unexpected due to its classification as a super antigen. PBL proliferation is an important part of the immune response both for generating antigen reactive cells and induction of numerous modulating cytokines. In the newborn these processes are essential as a building block for development of an optimal immune response and provide a protective host defense barrier against diseases associated with the neonatal gut.

Table 1 - Effect of CCP on Fresh Human Leukocyte Cultures

Peptide	Peptide Conc. µg/ml	Slide No.	Microscope 3 plus to 0	Mitogenic Activity CPM
SEQ ID NO:1	100	1	+++	1259
	10	2	++	4556
	1.0	3	+	4829
	0.1	4	+/-	3339
SEQ ID NO:7	100	5	++	1383
	10	6	+	3478
	1.0	7	+/-	2290
	0.1	8	-	584
SEQ ID NO:8	100		-	2039
	10	9	-	1810
	1.0	10	+++	1527
	0.1	11	++	2177
SEQ ID NO:3	100	ND	-	469
	10	ND	-	819
	1.0	ND	-	3323
	0.1	ND	-	86
SEQ ID NO:2	100	ND	-	29
	10	12	-	2989
	1.0	13	++	4809
	0.1	14	+/-	3578
SEQ ID NO:4	100	15	+	2667
	10	16	+	4915
	1.0	ND	-	4050
	0.1	ND	-	3523
SEQ ID NO:5	100	ND	-	1762
	10	ND	-	3304
	1.0	ND	-	1938
	0.1	ND	-	1630
SEQ ID NO:6	100	ND	-	748
	10	ND	-	3069
	1.0	ND	-	1375
	0.1	ND	-	1171
SEQ ID NO:31	100	23	+++	2039
	10	24	++	200
	1.0	25	+	901
	0.1	26	-	1875
Colostrinin	10	20	++	2470
	1.0	21	+	1614
	0.1	22	-	2535
Colostrum	100	17	++	1094
	10	18	-	2991

	1.0 0.1	19 ND	- -	3320 2717
SEA	.02	ND	++++	6554
Control		27	-	461

ND = not done
+++ = strong induction of lymphoblasts and/or monocytes
++ = medium induction of lymphoblasts and/or monocytes
+ = low induction of lymphoblasts and/or monocytes
+/- = some induction of lymphoblasts and/or monocytes
- = same as control
Mitogenic Activity = CPM above control as determined by 24-hour ^{3}H-thymidine incorporation.

Cytokine studies: Colostrinin has previously been shown in the literature to induce IFN-γ and TNF-α, as has Val-Glu-Ser-Tyr-Val-Pro-Leu-Phe-Pro (SEQ ID NO:31), which is disclosed in International Publication No. WO-A-98/1447. Thus, studies were done to investigate the individual peptides.

Cytokine concentrations were also determined from cells following 72 hours of incubation with concentrations of colistrinin and its constituent peptides (CCP) ranging from 100 to 0.1 μg/ml, and colostrum and high- or low-iron baby formula (Enfamil) at various dilutions. Supernatant fluids were then subjected to enzyme-linked immunosorbent assay (ELISA) for the following commercially available cytokines: interferon-gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), interleukin (IL)-4, IL-6, IL-10, and IL-12.

Table 2 represents the results of approximately 250 single assays. More specifically, in these studies it was found that many of the peptides including colostrinin induced IFN-γ and that the data corresponds with ^{3}H-thymidine incorporation (Tables 1 and 3). Interestingly the maximum cytokine inducing activity of many of the peptides was not diluted out until the 1.0 or 0.1 μg/ml concentrations of peptide were used (Shaded numbers in Table 2), or in the case of IFN-γ and TNF-α induction by SEQ ID NO:31 and SEQ ID NO:1, 0.1 μg/ml rather than higher concentrations. This finding may be consistent with a phasic response like those of hormones or of toxicity present in higher concentrations.

The ability to induce IFN-γ by some of the peptides decayed over time.

For example, SEQ ID NO:31 at 0.1 μg/ml at the beginning of the studies induced 324 pg IFN–γ/ml and in the last experiments induced no detectable levels. Although the peptides lost the IFN-γ inducing activity over a period of four months when stored in solution, some of the peptides were still able to induce TNF-α, IL-6, and IL-12, but the levels produced were somewhat lower than in the earlier studies. In contrast, induction of TNF-α and IL-10 by colostrinin and colostrum was still very high at this time. Thus, the complexed peptides making up colostrinin and colostrum may be more stable and/or combinations of peptides in colostrinin and colostrum may be more potent. Additional factors that may account for the variations of the peptides in these studies include: 1) natural variations in the immune state of the individuals donating the leukocytes, 2) the possibility that aggregation occurred in samples stored in PBS, thus reducing in effective number of molecules able to react, and 3) the possibility that the individual peptides may be subject to oxidative damage or some other inactivating process. The fact that the peptide, SEQ ID NO:8, which still induced IFN-γ in the last experiment (Example 3) was stored in 33% DMSO suggests an oxidative process or aggregation problem may be responsible for loss, or reduction of inducing activity in peptide samples stored in phosphate buffered saline (PBS). However, the samples in PBS appeared to be in solution at the time of the induction experiments.

Table 2. Cytokines induced in human leukocyte cultures stimulated with CCP, colostrum or commercial milk formulas.

PEPTIDE (Exp. #)	PEPTIDE CONCENTRATION (mg/ml)	IFN γ (pg/ml)	TNF-α (pg/ml)	IL-10 (pg/ml)
Example 1				
SEQ ID NO:1	100	54	478	168
	10	526	>1000	940
	1	584	>1000	1070
	0.1	236	722	696
SEQ ID NO:7	100	317	>1000	998
	10	409	>1000	1134
SEQ ID NO:8	100	419	>1000	860
	10	775	>1000	1643
	1	877	>1000	2223
	0.1	642	>1000	1350
SEQ ID NO:3	1	809	>1000	1611
	0.1	206	802	611
SEQ ID NO:2	100	372	>1000	754
	10	410	>1000	1063
	1	826	>1000	2092
	0.1	259	>1000	596
SEQ ID NO:4	10	794	>1000	1494
	1	723	>1000	1765
SEQ ID NO:5	100	559	>1000	756
	10	626	>1000	1158
SEQ ID NO:6	100	91	718	302
	10	621	>1000	1203
SEQ ID NO:31	100	371	804	4234
	10	107	370	1834
	1	118	651	242
	0.1	324	>1000	356

Colistrinin	10	888	>1000	1515
	1	878	>1000	1150
	0.1	156	760	451
Raw Colostrum	100	807	>1000	857
	10	530	>1000	1074
	1	934	>1000	1645
	0.1	192	848	391
Control		4	52	0
SEA		902	>1000	4676

Example 2

SEQ ID NO:18	100	4	24	36
SEQ ID NO:19	10	6	65	76
	1	463	>1000	502
SEQ ID NO:20	100	9	30	21
	10	31	118	101
SEQ ID NO:22	100	535	>1000	524
	10	539	985	409
	1	649	>1000	460
	0.1	147	636	207
SEQ ID NO:1	100	9	92	108
	10	14	99	129
	1	287	728	292
	0.1	576	>1000	397
SEQ ID NO:7	100	762.9	>1000	639
SEQ ID NO:2	100	980	>1000	646
	10	828	>1000	651
	1	914	>1000	1093
	0.1	281	685	348

	Enfamil	1:5	101	305	24
	Low Iron	1:10	167	406	443
	Enfamil	1:5	24	528	136
	with Iron	1:10	10	320	702
	Control		7	248	180
	SEA		901	>1000	2806
	Example 3				
	SEQ ID NO:1	100	6	110	0
		10	4	ND	ND
	SEQ ID NO:7	1	9	57	0
		0.1	6	ND	ND
	SEQ ID NO:8	10	8	20	0
		1	288	ND	ND
	SEQ ID NO:5	100	3	0	0
	Raw	100	5	11	0
	Colostrum	10	15	520	569
		1	0	ND	ND
		0.1	0	ND	ND
	Colostrinin	10	0	>1000	3662
		1	18	910	1839
		0.1	1	ND	ND
	SEQ ID NO:31	10	0	11	0
		1	0	90	0
		0.1	0	ND	ND
	SEQ ID NO:22	100	0	120	77.6
		10	0	60	0
		1	0	7	0
		0.1	0	ND	ND

Enfamil Low Iron	1:5	25	339	51
Enfamil with Iron	1:5	0	452	51
Control		0	0	0
SEA		700	>1000	2971
Example 4				
SEQ ID NO:1	100	0	73.3	0
SEQ ID NO:2	1	0	0	0
Colostrinin	10	0	1790	6.9
	1	0	1813	0
	0.1	ND	ND	ND
Raw Colostrum	100	0	1834	4.0
	10	0	31.2	0
	1	ND	ND	ND
Control		0	28.4	0
SEA		3.5	1927	13.4

Table 2. (cont.) Cytokines induced in human leukocyte cultures stimulated with CCP, colostrum or commercial milk formulas.

PEPTIDE (Exp. #)	PEPTIDE CONCENTRATION (mg/ml)	IL-4 (pg/ml)	IL-6 (pg/ml)	IL-12 (pg/ml)
Example 1				
SEQ ID NO:1	100	0	235.4	0
	10	0	934.8	0
	1	0	675.3	0
	0.1	0	497.1	0
SEQ ID NO:7	100	0	291.3	0
	10	0	645.4	0
SEQ ID NO:8	100	0	1076	0
	10	0	1024	0
	1	0	1013	0
	0.1	0	533.6	0
SEQ ID NO:3	1	0	620.5	0
	0.1	0	107	0
SEQ ID NO:2	100	0	258.6	0
	10	0	551.3	0
	1	0	1205	0
	0.1	0	325	0
SEQ ID NO:4	10	0	1718	0
	1	0	744.4	0
SEQ ID NO:5	100	0	98.2	0
	10	0	750	0
SEQ ID NO:6	100	0	63.3	0
	10	0	864.5	0

	SEQ ID NO:31	100	1.4	1489	0
		10	0	836.3	0
		1	0.4	489.9	0
		0.1	2.4	1635	0
	Colostrinin	10	0	1832	0
		1	1.9	1915	0
		0.1	0.4	430.1	0
	Raw Colostrum	100	0	1879	0
		10	0	602.2	0
		1	0	1055	0
		0.1	5.0	187.2	0
	Control		0	13.5	0
	SEA		4	1704	0

Example 2

	SEQ ID NO:18	100	0	142.4	0
	SEQ ID NO:19	10	0	549.7	0
		1	33.8	1552	0
	SEQ ID NO:20	100	0	50	0
		10	0.4	105.9	0
	SEQ ID NO:22	100	41.5	808.6	0
		10	32.7	503.2	0
		1	30.1	1005	0
		0.1	17.8	396.4	0
	SEQ ID NO:1	100	0	1471	0
		10	3.5	96.5	5.7
		1	26.6	626.6	0
		0.1	47.6	1385	0
	SEQ ID NO:7	100	24.5	1546	0
	SEQ ID NO:2	100	22.5	1292	33.5
		10	19.9	1516	0

		1	10.1	1886	9.9
		0.1	29.1	478.3	2.2
	Enfamil	1:5	0.9	1757	0
	Low Iron	1:10	4.0	1958	0
	Enfamil	1:5	0	1909	0
	with Iron	1:10	0	ND	0
	Control		0	183.5	0
	SEA		62.5	1769	54.8
	Example 3				
	SEQ ID NO:1	100	0	942.5	0
		10	ND	ND	ND
	SEQ ID NO:7	1	0	32.9	0
		0.1	ND	ND	ND
	SEQ ID NO:8	10	0	18.5	4.0
		1	ND	ND	ND
	SEQ ID NO:5	100	0	0	0
	Raw	100	0	0	0
	Colostrum	10	0	1853	1.6
		1	ND	ND	ND
		0.1	ND	ND	ND
	Colostrinin	10	0	2009	17.6
		1	0	1861	7.5
		0.1	ND	ND	ND
	SEQ ID NO:31	10	0	16.8	18.7
		1	0	722.9	0
		0.1	ND	ND	ND
	SEQ ID NO:22	100	6.0	1630	0
		10	0	46.7	0
		1	0	0	0

	0.1	ND	ND	ND
Enfamil Low Iron	1:5	0	1913	0
Enfamil with Iron	1:5	0.4	1953	0
Control		0	0	0
SEA		16.8	866.2	0

*SEQ ID NOs:1-8 and 31, Raw Colostrum, and Colostrinin were reconstituted on the same day.
*SEQ ID NOs:18, 19, 20, and 22 were reconstituted on the same day.

The relative abilities of the various peptides to induce cytokines are shown in Table 3. The peptides were ranked according to their abilities to induce the indicated cytokine by first comparing the raw numbers at the 0.1 μg/ml concentration followed by 1.0 μg/ml concentrations and then higher concentrations, i.e., 10 and 100 μg/ml. It can be noted that SEQ ID NOs:1, 8, 3, 2, and 31 were the best overall inducers in almost all cytokine and blood cell proliferation experiments. Peptides SEQ ID NOs:7, 4, and 5 were generally less effective as inducers. Colistrinin and colostrum ranked generally in the middle, however, only 1:5 and 1:10 dilutions of colostrum were used, thus actual comparison are not accurate since specific protein species present and their concentrations were not determined for colostrum. It is important to note, however, that colostrum contained substances that could induce cytokines in a similar fashion to colostrinin and its component peptides.

When the colostrinin constituent peptides having a β-casein precursor (SEQ ID NOs: 18, 19, 20, and 22) were compared to the initially tested SEQ ID NOs:1-8 and 31, the latter were better inducers. SEQ ID NO:22 was generally the best inducer of those peptides having a β-casein precursor. It was also found that Enfamil low iron baby formula induced higher levels of cytokines than the Enfamil high iron formula.

Table 3. Relative abilities of the various peptides to induce cytokines and proliferation

	Ex. 1	Ex. 2	Ex. 1	Ex. 1	Ex. 1	Ex. 2	Ex. 1
Rank	IFN-γ	IFN-γ	Micro. Resp.	Prolif. Resp.	TNF-α	TNF-α	IL-10
1	SEQ ID NO:8	SEQ ID NO:1	SEQ ID NO:8	SEQ ID NO:2	SEQ ID NO:2**	SEQ ID NO:2	SEQ ID NO:8
2	SEQ ID NO:31	SEQ ID NO:2	SEQ ID NO:2	SEQ ID NO:1	SEQ ID NO:8	SEQ ID NO:1	SEQ ID NO:1
3	SEQ ID NO:2	SEQ ID NO:7	SEQ ID NO:31	SEQ ID NO:4	SEQ ID NO:31	SEQ ID NO:7	SEQ ID NO:3
4	SEQ ID NO:1	SEQ ID NO:22	SEQ ID NO:1	Colostrum	Colostrum	SEQ ID NO:22	SEQ ID NO:2
5	SEQ ID NO:3	SEQ ID NO:19	SEQ ID NO:7	Colostrinin	Colostrinin	SEQ ID NO:19	Colostrinin
6	Colistrinin	SEQ ID NO:20	Colostrinin	SEQ ID NO:8	SEQ ID NO:3	SEQ ID NO:20	Colostrum
7	Colustrum	SEQ ID NO:18	Colostrum	SEQ ID NO:31	SEQ ID NO:1	SEQ ID NO:18	SEQ ID NO:31
8	SEQ ID NO:4		SEQ ID NO:3	SEQ ID NO:5	SEQ ID NO:5		SEQ ID NO:4
9	SEQ ID NO:5		SEQ ID NO:4	SEQ ID NO:6	SEQ ID NO:7	Low Enfamil	SEQ ID NO:7
9	SEQ ID NO:6		SEQ ID NO:5	SEQ ID NO:7	SEQ ID NO:4	High Enfamil	SEQ ID NO:5
10	SEQ ID NO:7		SEQ ID NO:6	SEQ ID NO:3	SEQ ID NO:6		SEQ ID NO:6

* SEQ ID NO:7 < 2 fold difference in titer
** All good inducers
*** No difference in titer

Table 3. (Cont.) Relative abilities of the various peptides to induce cytokines and proliferation

Rank	Ex. 2 IL-10	Ex. 1 IL-4	Ex. 2 IL-4	Ex. 1 IL-6	Ex. 2 IL-6	Ex. 1 IL-12	Ex. 2 IL-12
1	SEQ ID NO:2	Colostrum	SEQ ID NO:1	SEQ ID NO:31	Control	All neg.	SEQ ID NO:2
2	SEQ ID NO:7	Colostrinin	SEQ ID NO:2	SEQ ID NO:8			SEQ ID NO:1
3	SEQ ID NO:1	SEQ ID NO:31	SEQ ID NO:22	SEQ ID NO:1			
4	SEQ ID NO:19		SEQ ID NO:19	Colostrinin			
5	SEQ ID NO:22		SEQ ID NO:7	SEQ ID NO:2			
6	SEQ ID NO:20		Low Enfamil	Colostrum			
7	SEQ ID NO:18			SEQ ID NO:3			
8				SEQ ID NO:4			
9	Low Enfamil			SEQ ID NO:6			
9	High Enfamil			SEQ ID NO:5			
10				SEQ ID NO:7			

* SEQ ID NO:7 < 2 fold difference in titer
** All good inducers
*** No difference in titer

Although the invention has been disclosed with reference to its preferred embodiments, from reading this description those of skill in the art may appreciate changes and modification that may be made which do not depart from the scope and spirit of the invention as described above and claimed hereafter. All references, patents, and patent applications cited herein are incorporated herein by reference in their entirety as if individually incorporated.

Sequence Listing Free Text

The following are all synthetic peptide sequences.

SEQ ID NO:1	MQPPPLP
SEQ ID NO:2	LQTPQPLLQVMMEPQGD
SEQ ID NO:3	DQPPDVEKPDLQPFQVQS
SEQ ID NO:4	LFFFLPVVNVLP
SEQ ID NO:5	DLEMPVLPVEPFPFV
SEQ ID NO:6	MPQNFYKLPQM
SEQ ID NO:7	VLEMKFPPPPQETVT
SEQ ID NO:8	LKPFPKLKVEVFPFP
SEQ ID NO:9	VVMEV
SEQ ID NO:10	SEQP
SEQ ID NO:11	DKE
SEQ ID NO:12	FPPPK
SEQ ID NO:13	DSQPPV
SEQ ID NO:14	DPPPPQS
SEQ ID NO:15	SEEMP
SEQ ID NO:16	KYKLQPE
SEQ ID NO:17	VLPPNVG
SEQ ID NO:18	VYPFTGPIPN
SEQ ID NO:19	SLPQNILPL
SEQ ID NO:20	TQTPVVVPPF
SEQ ID NO:21	LQPEIMGVPKVKETMVPK

SEQ ID NO:22	HKEMPFPKYPVEPFTESQ
SEQ ID NO:23	SLTLTDVEKLHLPLPLVQ
SEQ ID NO:24	SWMHQPP
SEQ ID NO:25	QPLPPTVMFP
SEQ ID NO:26	PQSVLS
SEQ ID NO:27	LSQPKVLPVPQKAVPQRDMPIQ
SEQ ID NO:28	AFLLYQE
SEQ ID NO:29	RGPFPILV
SEQ ID NO:30	ATFNRYQDDHGEEILKSL
SEQ ID NO:31	VESYVPLFP
SEQ ID NO:32	FLLYQEPVLGPVR
SEQ ID NO:33	LNF
SEQ ID NO:34	MHQPPQPLPPTVMFP

We claim:

1. A method of inducing a cytokine in a cell, the method comprising contacting the cell with an immunological regulator under conditions effective to induce a cytokine, wherein the immunological regulator is selected from the group of MQPPPLP (SEQ ID NO:1), LQTPQPLLQVMMEPQGD (SEQ ID NO:2), DQPPDVEKPDLQPFQVQS (SEQ ID NO:3), LFFFLPVVNVLP (SEQ ID NO:4), DLEMPVLPVEPFPFV (SEQ ID NO:5), MPQNFYKLPQM (SEQ ID NO:6), VLEMKFPPPPQETVT (SEQ ID NO:7), LKPFPKLKVEVFPFP (SEQ ID NO:8), VVMEV (SEQ ID NO:9), SEQP (SEQ ID NO:10), DKE (SEQ ID NO:11), FPPPK (SEQ ID NO:12), DSQPPV (SEQ ID NO:13), DPPPPQS (SEQ ID NO:14), SEEMP (SEQ ID NO:15), KYKLQPE (SEQ ID NO:16), VLPPNVG (SEQ ID NO:17), VYPFTGPIPN (SEQ ID NO:18), SLPQNILPL (SEQ ID NO:19), TQTPVVVPPF (SEQ ID NO:20), LQPEIMGVPKVKETMVPK (SEQ ID NO:21), HKEMPFPKYPVEPFTESQ (SEQ ID NO:22), SLTLTDVEKLHLPLPLVQ (SEQ ID NO:23), SWMHQPP (SEQ ID NO:24), QPLPPTVMFP (SEQ ID NO:25), PQSVLS (SEQ ID NO:26), LSQPKVLPVPQKAVPQRDMPIQ (SEQ ID NO:27), AFLLYQE (SEQ ID NO:28), RGPFPILV (SEQ ID NO:29), ATFNRYQDDHGEEILKSL (SEQ ID NO:30), FLLYQEPVLGPVR (SEQ ID NO:32), LNF (SEQ ID NO:33), and MHQPPQPLPPTVMFP (SEQ ID NO:34), an active analog thereof, and combinations thereof, with the proviso that the immunological regulator is not VESYVPLFP (SEQ ID NO:31).

2. The method of claim 1 wherein the cell is present in a cell culture, a tissue, an organ, or an organism.

3. The method of claim 1 wherein the cell is a mammalian cell.

4. The method of claim 3 wherein the cell is a human cell.

5. The method of claim 1 wherein the immunological regulator is selected from the group of MQPPPLP (SEQ ID NO:1), LQTPQPLLQVMMEPQGD

(SEQ ID NO:2), DQPPDVEKPDLQPFQVQS (SEQ ID NO:3), LFFFLPVVNVLP (SEQ ID NO:4), DLEMPVLPVEPFPFV (SEQ ID NO:5), MPQNFYKLPQM (SEQ ID NO:6), VLEMKFPPPPQETVT (SEQ ID NO:7), LKPFPKLKVEVFPEP (SEQ ID NO:8),VYPFTGPIPN (SEQ ID NO:18), SLPQNILPL (SEQ ID NO:19), TQTPVVVPPF (SEQ ID NO:20), HKEMPFPKYPVEPFTESQ (SEQ ID NO:22), and combinations thereof.

6. A method for modulating an immune response in a cell, the method comprising contacting the cell with an immunological regulator under conditions effective to induce a cytokine, wherein the immunological regulator is selected from the group of MQPPPLP (SEQ ID NO:1), LQTPQPLLQVMMEPQGD (SEQ ID NO:2), DQPPDVEKPDLQPFQVQS (SEQ ID NO:3), LFFFLPVVNVLP (SEQ ID NO:4), DLEMPVLPVEPFPFV (SEQ ID NO:5), MPQNFYKLPQM (SEQ ID NO:6), VLEMKFPPPPQETVT (SEQ ID NO:7), LKPFPKLKVEVFPFP (SEQ ID NO:8), VVMEV (SEQ ID NO:9), SEQP (SEQ ID NO:10), DKE (SEQ ID NO:11), FPPPK (SEQ ID NO:12), DSQPPV (SEQ ID NO:13), DPPPPQS (SEQ ID NO:14), SEEMP (SEQ ID NO:15), KYKLQPE (SEQ ID NO:16), VLPPNVG (SEQ ID NO:17), VYPFTGPIPN (SEQ ID NO:18), SLPQNILPL (SEQ ID NO:19), TQTPVVVPPF (SEQ ID NO:20), LQPEIMGVPKVKETMVPK (SEQ ID NO:21), HKEMPFPKYPVEPFTESQ (SEQ ID NO:22), SLTLTDVEKLHLPLPLVQ (SEQ ID NO:23), SWMHQPP (SEQ ID NO:24), QPLPPTVMFP (SEQ ID NO:25), PQSVLS (SEQ ID NO:26), LSQPKVLPVPQKAVPQRDMPIQ (SEQ ID NO:27), AFLLYQE (SEQ ID NO:28), RGPFPILV (SEQ ID NO:29), ATFNRYQDDHGEEILKSL (SEQ ID NO:30), FLLYQEPVLGPVR (SEQ ID NO:32), LNF (SEQ ID NO:33), and MHQPPQPLPPTVMFP (SEQ ID NO:34), an active analog thereof, and combinations thereof, with the proviso that the immunological regulator is not VESYVPLFP (SEQ ID NO:31).

7. The method of claim 6 wherein the cell is present in a cell culture, a tissue, an organ, or an organism.

8. The method of claim 6 wherein the cell is a mammalian cell.

9. The method of claim 8 wherein the cell is a human cell.

10. The method of claim 6 wherein the immunological regulator is selected from the group of MQPPPLP (SEQ ID NO:1), LQTPQPLLQVMMEPQGD (SEQ ID NO:2), DQPPDVEKPDLQPFQVQS (SEQ ID NO:3), LFFFLPVVNVLP (SEQ ID NO:4), DLEMPVLPVEPFPFV (SEQ ID NO:5), MPQNFYKLPQM (SEQ ID NO:6), VLEMKFPPPPQETVT (SEQ ID NO:7), LKPFPKLKVEVFPEP (SEQ ID NO:8), VYPFTGPIPN (SEQ ID NO:18), SLPQNILPL (SEQ ID NO:19), TQTPVVVPPF (SEQ ID NO:20), HKEMPFPKYPVEPFTESQ (SEQ ID NO:22), and combinations thereof.

11. A method for modulating an immune response in a patient, the method comprising administering to the patient an immunological regulator under conditions effective to induce a cytokine, wherein the immunological regulator is selected from the group of MQPPPLP (SEQ ID NO:1), LQTPQPLLQVMMEPQGD (SEQ ID NO:2), DQPPDVEKPDLQPFQVQS (SEQ ID NO:3), LFFFLPVVNVLP (SEQ ID NO:4), DLEMPVLPVEPFPFV (SEQ ID NO:5), MPQNFYKLPQM (SEQ ID NO:6), VLEMKFPPPPQETVT (SEQ ID NO:7), LKPFPKLKVEVFPFP (SEQ ID NO:8), VVMEV (SEQ ID NO:9), SEQP (SEQ ID NO:10), DKE (SEQ ID NO:11), FPPPK (SEQ ID NO:12), DSQPPV (SEQ ID NO:13), DPPPPQS (SEQ ID NO:14), SEEMP (SEQ ID NO:15), KYKLQPE (SEQ ID NO:16), VLPPNVG (SEQ ID NO:17), VYPFTGPIPN (SEQ ID NO:18), SLPQNILPL (SEQ ID NO:19), TQTPVVVPPF (SEQ ID NO:20), LQPEIMGVPKVKETMVPK (SEQ ID NO:21), HKEMPFPKYPVEPFTESQ (SEQ ID NO:22), SLTLTDVEKLHLPLPLVQ (SEQ ID NO:23), SWMHQPP (SEQ ID NO:24), QPLPPTVMFP (SEQ ID NO:25), PQSVLS (SEQ ID NO:26), LSQPKVLPVPQKAVPQRDMPIQ (SEQ ID NO:27), AFLLYQE (SEQ ID NO:28), RGPFPILV (SEQ ID NO:29), ATFNRYQDDHGEEILKSL (SEQ ID NO:30), FLLYQEPVLGPVR (SEQ ID NO:32), LNF (SEQ ID NO:33), and

MHQPPQPLPPTVMFP (SEQ ID NO:34), an active analog thereof, and combinations thereof, with the proviso that the immunological regulator is not VESYVPLFP (SEQ ID NO:31).

12. The method of claim 11 wherein the immunological regulator is selected from the group of MQPPPLP (SEQ ID NO:1), LQTPQPLLQVMMEPQGD (SEQ ID NO:2), DQPPDVEKPDLQPFQVQS (SEQ ID NO:3), LFFFLPVVNVLP (SEQ ID NO:4), DLEMPVLPVEPFPFV (SEQ ID NO:5), MPQNFYKLPQM (SEQ ID NO:6), VLEMKFPPPPQETVT (SEQ ID NO:7), LKPFPKLKVEVFPEP (SEQ ID NO:8), VYPFTGPIPN (SEQ ID NO:18), SLPQNILPL (SEQ ID NO:19), TQTPVVVPPF (SEQ ID NO:20), HKEMPFPKYPVEPFTESQ (SEQ ID NO:22), and combinations thereof.

13. The method of claim 11 wherein the immunological regulator is administered as part of a dietary supplement.

14. The method of claim 11 wherein the immunological regulator is administered topically.

15. The method of claim 11 wherein the patient is an animal.

16. The method of claim 15 wherein the patient is a human.

17. The method of claim 11 wherein the immune response is a specific immune response.

18. The method of claim 11 wherein the immune response is a nonspecific immune response.

19. The method of claim 11 wherein the immune response is the interferon response or antibody production.

20. A method for modulating blood cell proliferation, the method comprising contacting blood cells with a blood cell regulator selected from the group of colostrinin, a constituent peptide thereof, an analog thereof, and combinations thereof, under conditions effective to change the number of blood cells.

21. The method of claim 20 wherein the blood cells are present in a cell culture or an organism.

22. The method of claim 20 wherein the blood cells are mammalian cells.

23. The method of claim 22 wherein the blood cells are human cells.

24. The method of claim 22 wherein the blood cells are increased in number.

25. The method of claim 24 wherein the blood cells are differentiated.

26. The method of claim 22 wherein the blood cell regulator is a constituent peptide of colostrinin.

27. The method of claim 26 wherein the blood cell regulator is selected from the group of MQPPPLP (SEQ ID NO:1), LQTPQPLLQVMMEPQGD (SEQ ID NO:2), DQPPDVEKPDLQPFQVQS (SEQ ID NO:3), LFFFLPVVNVLP (SEQ ID NO:4), DLEMPVLPVEPFPFV (SEQ ID NO:5), MPQNFYKLPQM (SEQ ID NO:6), VLEMKFPPPPQETVT (SEQ ID NO:7), LKPFPKLKVEVFPFP (SEQ ID NO:8), VVMEV (SEQ ID NO:9), SEQP (SEQ ID NO:10), DKE (SEQ ID NO:11), FPPPK (SEQ ID NO:12), DSQPPV (SEQ ID NO:13), DPPPPQS (SEQ ID NO:14), SEEMP (SEQ ID NO:15), KYKLQPE (SEQ ID NO:16), VLPPNVG (SEQ ID NO:17), VYPFTGPIPN (SEQ ID NO:18), SLPQNILPL (SEQ ID NO:19), TQTPVVVPPF (SEQ ID NO:20), LQPEIMGVPKVKETMVPK (SEQ ID NO:21), HKEMPFPKYPVEPFTESQ (SEQ ID NO:22), SLTLTDVEKLHLPLPLVQ (SEQ ID NO:23), SWMHQPP

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28. The method of claim 27 wherein the blood cell regulator is selected from the group of MQPPPLP (SEQ ID NO:1), LQTPQPLLQVMMEPQGD (SEQ ID NO:2), DQPPDVEKPDLQPFQVQS (SEQ ID NO:3), LFFFLPVVNVLP (SEQ ID NO:4), DLEMPVLPVEPFPFV (SEQ ID NO:5), MPQNFYKLPQM (SEQ ID NO:6), VLEMKFPPPPQETVT (SEQ ID NO:7), LKPFPKLKVEVFPEP (SEQ ID NO:8), VYPFTGPIPN (SEQ ID NO:18), SLPQNILPL (SEQ ID NO:19), TQTPVVVPPF (SEQ ID NO:20), HKEMPFPKYPVEPFTESQ (SEQ ID NO:22), and combinations thereof.

29. A method for modulating blood cell proliferation in a patient, the method comprising administering to the patient a blood cell regulator selected from the group of colostrinin, a constituent peptide thereof, an analog thereof, and combinations thereof, under conditions effective to change the number of blood cells.

30. The method of claim 29 wherein the patient is a human.

31. The method of claim 29 wherein the blood cells are increased in number.

32. The method of claim 31 wherein the blood cells are differentiated.

33. The method of claim 29 wherein the blood cell regulator is a constituent peptide of colostrinin.

34. The method of claim 33 wherein the blood cell regulator is selected from the group of MQPPPLP (SEQ ID NO:1), LQTPQPLLQVMMEPQGD (SEQ ID NO:2), DQPPDVEKPDLQPFQVQS (SEQ ID NO:3), LFFFLPVVNVLP (SEQ ID NO:4), DLEMPVLPVEPFPFV (SEQ ID NO:5), MPQNFYKLPQM (SEQ ID NO:6), VLEMKFPPPPQETVT (SEQ ID NO:7), LKPFPKLKVEVFPFP (SEQ ID NO:8), VVMEV (SEQ ID NO:9), SEQP (SEQ ID NO:10), DKE (SEQ ID NO:11), FPPPK (SEQ ID NO:12), DSQPPV (SEQ ID NO:13), DPPPPQS (SEQ ID NO:14), SEEMP (SEQ ID NO:15), KYKLQPE (SEQ ID NO:16), VLPPNVG (SEQ ID NO:17), VYPFTGPIPN (SEQ ID NO:18), SLPQNILPL (SEQ ID NO:19), TQTPVVVPPF (SEQ ID NO:20), LQPEIMGVPKVKETMVPK (SEQ ID NO:21), HKEMPFPKYPVEPFTESQ (SEQ ID NO:22), SLTLTDVEKLHLPLPLVQ (SEQ ID NO:23), SWMHQPP (SEQ ID NO:24), QPLPPTVMFP (SEQ ID NO:25), PQSVLS (SEQ ID NO:26), LSQPKVLPVPQKAVPQRDMPIQ (SEQ ID NO:27), AFLLYQE (SEQ ID NO:28), RGPFPILV (SEQ ID NO:29), ATFNRYQDDHGEEILKSL (SEQ ID NO:30), VESYVPLFP (SEQ ID NO:31), FLLYQEPVLGPVR (SEQ ID NO:32), LNF (SEQ ID NO:33), and MHQPPQPLPPTVMFP (SEQ ID NO:34), an active analog thereof, and combinations thereof.

35. The method of claim 34 wherein the blood cell regulator is selected from the group of MQPPPLP (SEQ ID NO:1), LQTPQPLLQVMMEPQGD (SEQ ID NO:2), DQPPDVEKPDLQPFQVQS (SEQ ID NO:3), LFFFLPVVNVLP (SEQ ID NO:4), DLEMPVLPVEPFPFV (SEQ ID NO:5), MPQNFYKLPQM (SEQ ID NO:6), VLEMKFPPPPQETVT (SEQ ID NO:7), LKPFPKLKVEVFPEP (SEQ ID NO:8),VYPFTGPIPN (SEQ ID NO:18), SLPQNILPL (SEQ ID NO:19), TQTPVVVPPF (SEQ ID NO:20), HKEMPFPKYPVEPFTESQ (SEQ ID NO:22), and combinations thereof.

36. A cytokine-inducing composition comprising a pharmaceutical carrier and an active agent selected from the group of MQPPPLP (SEQ ID NO:1), LQTPQPLLQVMMEPQGD (SEQ ID NO:2), DQPPDVEKPDLQPFQVQS (SEQ ID NO:3), LFFFLPVVNVLP (SEQ ID NO:4), DLEMPVLPVEPFPFV

(SEQ ID NO:5), MPQNFYKLPQM (SEQ ID NO:6), VLEMKFPPPPQETVT (SEQ ID NO:7), LKPFPKLKVEVFPFP (SEQ ID NO:8), VVMEV (SEQ ID NO:9), SEQP (SEQ ID NO:10), DKE (SEQ ID NO:11), FPPPK (SEQ ID NO:12), DSQPPV (SEQ ID NO:13), DPPPPQS (SEQ ID NO:14), SEEMP (SEQ ID NO:15), KYKLQPE (SEQ ID NO:16), VLPPNVG (SEQ ID NO:17), VYPFTGPIPN (SEQ ID NO:18), SLPQNILPL (SEQ ID NO:19), TQTPVVVPPF (SEQ ID NO:20), LQPEIMGVPKVKETMVPK (SEQ ID NO:21), HKEMPFPKYPVEPFTESQ (SEQ ID NO:22), SLTLTDVEKLHLPLPLVQ (SEQ ID NO:23), SWMHQPP (SEQ ID NO:24), QPLPPTVMFP (SEQ ID NO:25), PQSVLS (SEQ ID NO:26), LSQPKVLPVPQKAVPQRDMPIQ (SEQ ID NO:27), AFLLYQE (SEQ ID NO:28), RGPFPILV (SEQ ID NO:29), ATFNRYQDDHGEEILKSL (SEQ ID NO:30), FLLYQEPVLGPVR (SEQ ID NO:32), LNF (SEQ ID NO:33), and MHQPPQPLPPTVMFP (SEQ ID NO:34), an active analog thereof, and combinations thereof, with the proviso that the active agent is not VESYVPLFP (SEQ ID NO:31).

SEQUENCE LISTING

<110> THE UNIVERSITY OF TEXAS SYSTEM
REGEN THERAPEUTICS PLC
STANTON, G. John
HUGHES, Thomas K.
BOLDOGH, Istvan
GEORGIADES, Jerzy

<120> USE OF COLOSTRININ, CONSTITUENT PEPTIDES THEREOF, AND ANALOGS THEREOF FOR INDUCING CYTOKINES

<130> 265.00230202

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<210> 34
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<213> Artificial Sequence

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<223> Description of Artificial Sequence: synthetic peptide

<400> 34
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1 5 10 15

INTERNATIONAL SEARCH REPORT

Int nal Application No
PCT/US 00/22775

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, MEDLINE, CHEM ABS Data, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 14473 A (JANUSZ MARIN ;LISOWSKI JOZEF (PL); DUBOWSKA INGLOT ANNA (PL); HIRS) 9 April 1998 (1998-04-09) See especially page 19, lines 31-37	20-26, 29-33
E	WO 00 75173 A (REGEN THERAPEUTICS PLC ;GEORGIADES JERZY A (US)) 14 December 2000 (2000-12-14) the whole document	1-19,36
E	WO 01 11937 A (REGEN THERAPEUTICS PLC ;BOLDOGH ISTVAN (US); STANTON G JOHN (US);) 22 February 2001 (2001-02-22) the whole document	1-36

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 June 2001	22/06/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Groenendijk, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/US 00/22775

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	INGLOT A D ET AL: "COLOSTRININE: A PROLINE-RICH POLYPEPTIDE FROM OVINE COLOSTRUM IS A MODEST CYTOKINE INDUCET IN HUMAN LEUKOCYTES" ARCHIVUM IMMUNOLOGIAE ET THERAPIAE EXPERIMENTALIS,PL,POLISH ACADEMY OF SCIENCES, WROCLAW, vol. 44, no. 4, 1 August 1996 (1996-08-01), pages 215-224, XP002055880 ISSN: 0004-069X cited in the application the whole document	1-36

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ial Application No
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		AU 4565197 A	24-04-1998
		BR 9712259 A	25-01-2000
		CN 1238782 A	15-12-1999
		EP 0932623 A	04-08-1999
		GB 2352176 A,B	24-01-2001
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		HU 9904368 A	28-06-2000
		JP 2001501929 T	13-02-2001
		PL 332632 A	27-09-1999
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WO 0111937 A	22-02-2001	NONE	